Original Article

EVALUATION OF THE REDUCTION OF NASOPHARYNGEAL VIRAL LOAD SECONDARY TO THE USE OF NASAL IRRIGATIONS USING THE NASIR © SYSTEM IN PATIENTS WITH SARS-COV-2: PRELIMINARY STUDIES

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All authors critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

ABSTRACT

BACKGROUND & AIMS: Since the outbreak of the COVID-19 pandemic, it has been widely hypothesised that saline nasal irrigations (SNIs) could reduce nasopharyngeal viral load and patients' infectiousness, preventing or slowing down the transmission of SARS-CoV-2. However, no previous studies had systematically evaluated changes in nasopharyngeal viral load. We evaluated how nasopharyngeal viral load changes over time following or not a SNI.
METHODS: Sixteen consecutive tested positive for SARS-CoV-2 infection were recruited. Patients were randomly divided into two groups: the experimental group(n=10) and the control group(n=6). The experimental group performed SNI with the Nasir® device. The control group didn't performed any SNI. Both groups underwent serial nasal swabs. The cycle threshold (Ct) values, indirect indexes of viral load, were recorded for all target genes and the average Ct value for each sample was used for analysis.
RESULTS: The differences of the average Ct values among the two study groups by time of sample collection were not statistically significant.
CONCLUSION: Although it was widely hypothesised that SNIs were promising methods in decreasing nasopharyngeal viral load, they cannot be considered effective in reducing patients' contagiousness and preventing SARS-CoV-2 transmission**.** Further studies**,** including on SNIs performed with additives with virucidal properties, are needed to verify the possibility of lowering viral load. Pending adequate vaccination coverage and effective therapies, non-pharmaceutical interventions (NPI), such as social distancing and use of PPEs, are still needed to reduce the risk of virus transmission.

KEY WORDS: SARS-CoV-2; COVID-19; Nasal irrigation; Nasopharyngeal viral load; Nasal Swab.

BACKGROUND & AIMS

The unprecedented outbreak of the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is currently a global concern. The lack of knowledge and incomplete understanding of COVID-19 limit current advances in research, product development and therapeutic strategies[1]. The absence of effective therapies, pending adequate vaccination coverage, has forced the entire population to implement several public health measures to prevent or slow down the transmission of SARS-CoV-2, including social distancing, hand hygiene, environmental disinfection and use of personal protective equipment (PPE)[2].

Since SARS-CoV-2 is known to transmit through airborne spread via respiratory droplets, the nasal epithelium and nasopharyngeal mucosa are key portals of entry, attachment, localization and replication of the virus. In this context, it has been hypothesised that nasal washes could confer protection against this virus and its spreading[3]. As a matter of fact, saline nasal Irrigations (SNIs) are non-pharmacological practices known to remove antigens, biofilms, bacteria, and viruses from nasal mucosa and improve mucociliary activity. Moreover, SNIs elicit the innate antiviral immunity of the nasal mucosa cells[4,5]. Therefore, several studies have suggested the potential usefulness of nasal washes with or without additives, such as povidone-iodine or Angiotensin-Converting Enzyme-2, in patients affected by Covid-19[6–8]. However, to our best knowledge, no studies have been conducted systematically to verify if nasal irrigations are effective in reducing the nasopharyngeal viral load of SARS-CoV-2 and, thus affecting the risk of transmission.

Based on this background, the aim of our study was to evaluate the effects of nasal irrigation on SARS-CoV-2 nasopharyngeal viral load, using the Nasir® device.

METHODS

Sixteen consecutive patients admitted to the COVID Department of the University Hospitals of Bari and Foggia from 9 April 2021 to 7 May 2021 were recruited for this observational study. Specific inclusion criteria were arranged as follows: genders: both; pathology: documented SARS-CoV-2 infection, assessed by at least one positive molecular test for SARS-CoV-2 and one positive nasopharyngeal (NP) swab. Specific exclusion criteria were clinical conditions that prevented the correct execution of SNIs.

Patients were randomly divided into two groups. The experimental group (n=10, labelled “NASIR patients”) performed SNI using the Nasir® device[9]. In particular, the Nasir® system consists of a sac (250 mL) of premixed sterile saline isotonic solution and a delivery system with a 60-cm tube, which generates a standard irrigation pressure of 0.058 Pa, adjustable with a regulator to improve the tolerability of the device. The irrigator tip, which is in continuity with the delivery system, dilates the nasal valve and distributes the solution to all portions of the nasal cavity. The control group (n=6, labelled “control group”) did not perform any SNI.

In order to evaluate the changes in the SARS-CoV-2 nasopharyngeal viral load, patients assigned to the experimental group underwent a first molecular NP swab immediately prior to nasal irrigation (T0), carried out with Nasir® device. The NP swab was then repeated 1 (T1), 6 (T2) and 12 (T3) hours from T0.

The control group also underwent a first molecular NP swab at time T0, repeated after 1 (T1), 6 (T2) and 12 (T3) hours.

NP swabs were performed following a standardised procedure. Molecular test was performed using a three-target (N, ORF1ab, and S genes) commercial multiplex real-time PCR assay from Thermo Fisher Scientific (TaqPath RT-PCR COVID-19 Assay)[10]. Results were interpreted according to the manufacturer's instructions. For each sample, the cycle threshold (Ct) values were recorded for all target genes and the average Ct value for each sample was used for analysis, since the Ct values can be considered as an indirect index of viral load[11].

Data analysis was performed using R Studio software (RStudio, Northern Ave, Boston, MA, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA). To compare differences between the average of Ct values in NASIR patient and control group, a T-test was used.

The study was approved by the Ethic Committee of the Policlinico University Hospital of Bari (number 6854). All procedures were carried out in accordance with the guidelines for research on human subjects of the Declaration of Helsinki, as revised in 2013. Informed written consent was obtained from all subjects involved in the study.

RESULTS

Overall, 16 patients were enrolled for the study. All patients were subjected to NPS at hospital admission to confirm the SARS-CoV-2 infection. The mean age was 63 years (range: 49-78) and 81.3% (13/16) were male. The demographic and clinical characteristics by patient group are shown in *Table I*. The average of Ct values of target genes at T0, T1, T2 and T3 by patient group are reported in *Figure 1*. The differences of the average Ct values among the two study groups by time of sample collection were not statistically significant (*Figure 1*).

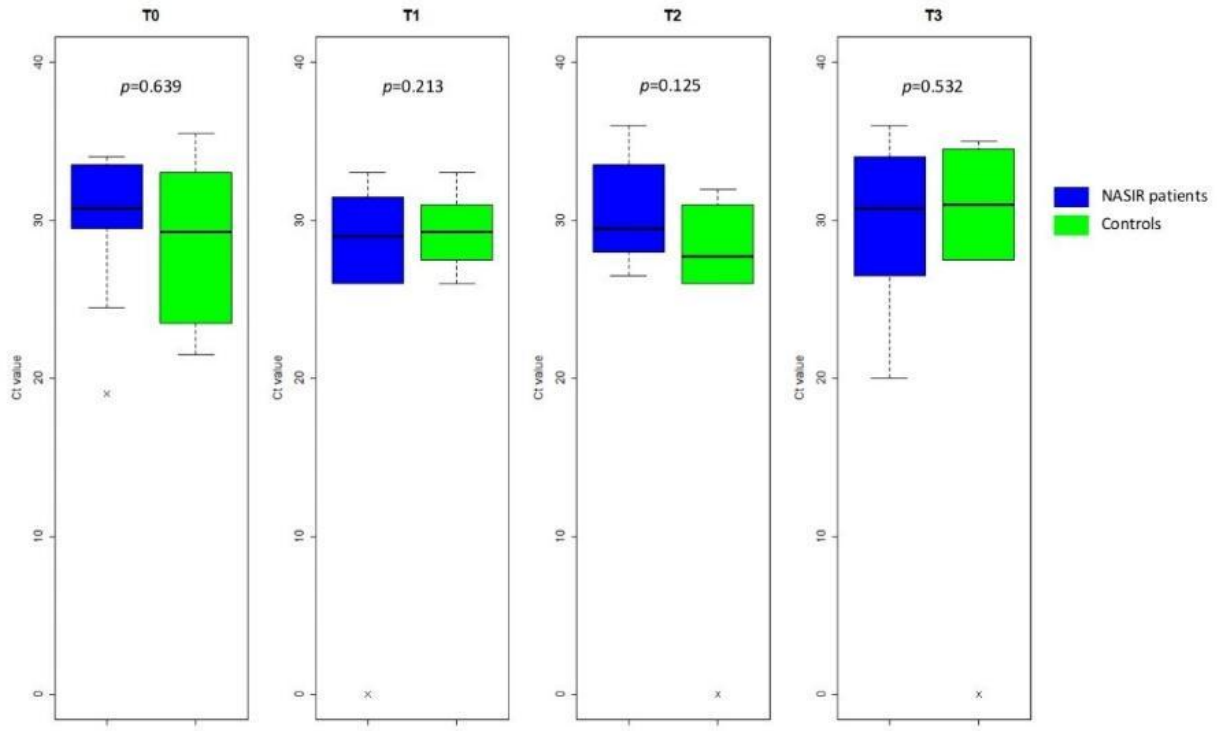

Figure 1. Boxplots of average Ct values of the NASIR and control patient groups by time of sample collection (T0, T1, T2, T3).

Table I. Demographic and clinical characteristics by patient group.

	Nasir group (N=10) N (%)	**Control group (N=6)** N (%)
Sex		
Male	8 (80.0)	5 (83.4)
Female	2 (20.0)	1 (16.6)
Age (mean)	61	65
Comorbidity	7 (70.0)	4 (66.7)
Respiratory support (O_2, HFNC, NIV*)	10 (100%)	6 (100%)
Time from first positive NPS (days)	0-8	0-12

*O_2= Oxygen

HFNC= High-flow nasal cannula oxygen

NIV= Non Invasive Ventilation

DISCUSSION

SARS-CoV-2 enters host cells through the interaction between the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, located on the surface of the viral particle, and its receptor on the surface of human cells, the angiotensin I converting enzyme 2 (ACE-2) receptor, with the help of transmembrane protease serine 2 (TMPRSS2) [12]. In particular, while ACE-2 receptor mediates cellular entry, TMPRSS2 favours SARS-CoV-2 entry by cleaving the viral spike protein into a conformational form necessary for membrane fusion. Interestingly, ACE2 expression in the nasopharynx is greater than in the alveolar tissue. Furthermore, TMPRSS2 expression also occurs more stably in the upper airways and alternative enzymes, such as cathepsin B/L or furin, may play its role in viral infection interchangeably[13]. This explains, although the pathophysiological mechanisms of COVID-19 are not yet fully understood, why the initiation of SARS-CoV-2 infection occurs in the upper respiratory tract. It is worth mentioning that the level of viral replication in the nasopharynx strongly correlates with the likelihood of transmission, which is higher in the first week[14]. On the contrary, the clinical manifestations of SARS-CoV-2 infection, which range from asymptomatic to severe acute respiratory distress syndrome (ARDS) and life-threatening multi-system organ failure, do not correlate with nasopharyngeal viral load[15,16].

Since reduced viral loads suggest potentially lower infectiousness, we hypothesized that SNIs could prevent or slow down SARS-CoV-2 transmission and, therefore, constitute a useful public health measure. As a matter of fact, nasal irrigations, unlike nasal sprays, reach the nasopharynx, which is the primary site of SARS-CoV-2 replication, and ensure proper cleansing by removing antigens, inflammatory mediators, and microorganisms such as bacteria and viruses [17,18]. Moreover, SNIs improve mucociliary clearance, which is impaired by SARS-CoV-2, elicit the innate antiviral immunity of the nasal mucosa and enhance the speed of wound healing in sinonasal cavities[19,20].

Therefore, we evaluated the changes in nasopharyngeal viral load over time, comparing the control group with the experimental group, which performed SNIs with the Nasir® device. During nasal irrigation, performed with the head bent downwards, the solution first flows through the nasal cavity closed by the irrigator, and then, after reaching the nasopharynx, crosses postero-anterior to the contralateral cavity. This allows to mechanically remove, with the saline solution, not only the pathogenic secretions but also dust, allergens and other contaminants, especially using large volume low pressure nasal irrigation [9].

Our study showed no differences in real-time PCR Ct values at time 0, after 1, 6, and 12 hours. Probably, these data confirm that viral shedding is not affected by any mechanical treatment. Virus particles are constantly expelled from the nasal mucosal cells, through cell lysis or by fusion of vacuoles containing virus with the cell plasma membrane. In fact, a recent study has shown that, since cells aren't infected at the same time, there is a desynchronized virus infection and thus a variability in the stage of infection in the mucosa[21]. Therefore, even after mechanical washing of the nasal cavities and nasopharynx, the cells continue to release viral particles that continuously make the patient contagious. Although SNIs do not appear to reduce the viral load, we believe that they should be recommended in patients with COVID19, in order to prevent bacterial

overlap. In fact, large-volume low-pressure SNIs remove pathological secretions and reduce the endonasal bacterial load by acting on bacterial adhesiveness[22]. In this context, adding virucidal agents to the saline solution used for SNIs could be useful for reducing viral load too. In fact, a recent study showed that povidone-iodine (PVP-I), an antiseptic agent with excellent virucidal (99.99%) properties, should be used to prevent COVID-19. However, this study has some important limitations, since the nasopharyngeal clearance of SARS-CoV-2 was tested only after single application of PVP-I via nasal spray or nasal irrigation at different concentrations and the viral quantification was not performed before and after the intervention[23].

CONCLUSIONS

Identifying effective prevention and treatment strategies against SARS-Cov-2 infection remains complex and challenging. On the basis of the findings of the present study, although it was hypothesised that SNIs were promising methods in decreasing nasopharyngeal viral load, they cannot be considered effective in reducing patients' contagiousness and preventing SARS-CoV-2 transmission. Therefore, non-pharmaceutical interventions (NPI), such as social distancing and use of PPEs, are still needed to reduce the risk of virus transmission. Further studies with larger sample sizes on other non-mechanical treatments, including SNIs performed with additives with virucidal properties, are needed to verify the possibility of lowering viral load.

Conflict of Interest Statement:
The authors have no conflicts of interest to declare.

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